J Infect Chemother 20 (2014) 666–671

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Review article

Lactoferrin for prevention of common viral infections

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ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form
4 August 2014
Accepted 4 August 2014
Available online 30 August 2014

Keywords:
Lactoferrin
Virus
Norovirus
Gastroenteritis
Interferon
NK

ABSTRACT

Although lactoferrin has many biological functions, the host-protective effects against pathogenic microorganisms including bacteria, fungi, and viruses are regarded as one of the most important. Here, we review research on the protective role of lactoferrin administration against common viral infections. Many studies have shown the in vitro antiviral activity of lactoferrin against viral pathogens that cause common infections such as the common cold, influenza, gastroenteritis, summer cold, and herpes, where lactoferrin inhibits mainly viral attachment to the target cells. Recently, studies indicating the in vivo protective effects of lactoferrin by oral administration against common viral infections have been increasing. For instance, norovirus is an extremely important emerging human pathogen that causes a majority of gastroenteritis outbreaks worldwide that may be a target candidate for lactoferrin. Lactoferrin consumption reduced the incidence of noroviral gastroenteritis in children and a similar effect was observed in a wide range of ages in a preliminary survey. A recent in vitro study reported that lactoferrin inhibits both cellular attachment of the murine norovirus, a virus closely-related to the human norovirus, and viral replication in the cells by inducing antiviral cytokines interferon (IFN)-α/β. Lactoferrin administration also enhances NK cell activity and Th1 cytokine responses, which lead to protection against viral infections. In conclusion, lactoferrin consumption may protect the host from viral infections through inhibiting the attachment of a virus to the cells, replication of the virus in the cells, and enhancement of systemic immune functions.

1. Introduction

Lactoferrin, an 80-kDa iron-binding glycoprotein of the transferrin family, is a component of exocrine secretions such as milk and saliva, and is present in neutrophil granules [1]. Lactoferrin is thought to play a role in host defense and exhibits a diverse range of biological activities, including antimicrobial activities, antiviral activities, antioxidant activities, immunomodulation, modulation of cell growth, and binding of several bioactive compounds [2–4]. The first report on the antiviral effect of lactoferrin was in the studies conducted by Broxmeyer's group in the 1980s. They showed that lactoferrin affects the myelopoiesis of mice inoculated with a friend virus complex [5]. Then, they found that ip-injected lactoferrin improved the survival rate of mice infected with a friend virus complex [6]. In the 1990s, the target viruses for which lactoferrin was shown to exhibit antiviral activity were propagated to cytomegalovirus (CMV), herpes simplex virus (HSV), human immunodeficiency virus (HIV), hepatitis C virus (HCV), rotavirus, poliovirus (PV), respiratory syncytial virus (RSV) [7]. The author of this review article described that the antiviral effect of lactoferrin lies in the early phase of infection, preventing the entry of a virus into the host cells, either by blocking cellular receptors, or by direct binding to the virus particles [7]. In a recent review article by Berlutti, the hepatitis B virus (HBV), parainfluenza virus (PIV), alphavirus, hantavirus, human papillomavirus (HPV), feline calicivirus (FCV), adenovirus, enterovirus 71 (EV71), echovirus 6, influenza A virus, Japanese encephalitis virus, and tomato yellow leaf curl virus (TYLCV) were added as newly identified viruses which are inhibited by lactoferrin [8]. In this review, the authors described that lactoferrin may exert its antiviral effect not only in the early phase of surface interaction between virus and cell, but also intracellularly because the nuclear localization of lactoferrin in different epithelial human cells has been observed.

Recently investigations to study the effects of orally administered lactoferrin against virus infections in animals and humans have been performed. These studies suggested that lactoferrin

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http://dx.doi.org/10.1016/j.jiac.2014.08.003
1341-321X

consumption exerts some protective effect against common viral infections. Here, we review the studies regarding common viral infections including the common cold, influenza, viral gastroenteritis, summer cold, and herpes, both in vitro and in vivo effect by oral administration, and discuss the prophylactic potential of lactoferrin as a food component.

2. Common cold and influenza

The common cold and influenza are the most common viral infections and they occur in the respiratory tract. The in vitro effects of lactoferrin against viruses causing common infections are summarized in Table 1. Many viruses cause the common cold. Among common cold viruses, the antiviral activity of lactoferrin is reported against the respiratory syncytial (RS) virus [9–11] and parainfluenza virus [12]. The anti-influenza virus activity of lactoferrin is also reported against the influenza A virus H1N1, H3N2, and H5N1 (avian) [13–16].

Effects of orally administered lactoferrin on common viral infections are summarized in Table 2. A questionnaire survey of adult women revealed that consumption of lactoferrin-containing tablets decreases the incidence of common cold-like symptoms and gastroenteritis symptoms [17]. Another study reported that lactoferrin administration with milk immunoglobulin reduces the incidence of the common cold in humans [18]. On the other hand, lactoferrin did not show a favorable effect in an RS virus infection model of mice [19]. In a mouse influenza virus-infection model, lactoferrin feeding lowered lung inflammatory markers [20]. There is no report regarding the effects of lactoferrin on influenza in humans yet. NK cells recognize and destroy target cells infected by influenza or the parainfluenza virus [21] and the relationship between the frequency of the common cold and the activity of NK cells has been reported [22]. It has been shown that lactoferrin feeding enhances NK cell activity in patients with adenomatous colorectal polyps [23] and the NK cell number in mice [24]. Therefore, increased NK cell activity or number by lactoferrin may mediate at least partly the host protection against the common cold and influenza.

3. Viral gastroenteritis

Gastroenteritis caused by rotavirus and norovirus is a major illness prevalent in winter. Rotavirus causes gastroenteritis only in children. Norovirus is an extremely important emerging human pathogen that causes a majority of gastroenteritis outbreaks worldwide. The in vitro anti-rotavirus effects of lactoferrin have been reported [25,26] (Table 1). The human norovirus remains difficult to study, because there is a lack of cell cultures and animal models. Instead, feline calicivirus and murine norovirus, which can be cultured and share a number of biochemical properties, similar genomic organization and primary RNA sequences with human norovirus, have been used as a virus surrogate to study human norovirus. A study using feline calicivirus showed that bovine lactoferrin inhibits the viral infection of Crandell-Reese feline kidney cells by binding to the cells and lactoferricin B inhibits the infection by binding to the virus [27]. Bovine lactoferrin also decreased murine norovirus infection to murine macrophage cell line Raw264.7 through inhibition of the initial murine norovirus attachment to cells and the subsequent interference with murine norovirus replication [28]. The induction of antiviral cytokine interferon (IFN)-α/β expression by lactoferrin was involved in inhibition of viral replication in the infected cells. This is the first report that shows the inhibition of viral replication in the cells and the involvement of IFN-α/β in the antiviral effect of lactoferrin. It has already been reported that oral administration of lactoferrin induces IFN-α/β in the small intestine of mice [24,29]. From these findings, IFN-α/β may be a key mediator in the antiviral effects of orally administered lactoferrin and the deduced antiviral mechanism of lactoferrin was illustrated in Fig. 1.

The effects of the oral administration of lactoferrin against viral gastroenteritis, where rotavirus or norovirus was identified as a pathogen, have been reported (Table 2). In a study of rotaviral gastroenteritis in children, daily intake of bovine lactoferrin-containing products ameliorated the severity of the disease, although there was no significant benefit in reducing infection incidence [30]. The addition of recombinant human lactoferrin and lysozyme to a rice-based oral rehydration solution had beneficial effects on children with acute diarrhea in whom rotavirus was identified as a pathogen in 18–19% of stool samples [31]. The daily administration of lactoferrin tablets to children reduced the incidence of noroviral gastroenteritis [32]. Lactoferrin administration exhibited no decrease in diarrhea incidence, but decreased longitudinal prevalence and severity in children, where norovirus was isolated as a pathogen in 35% of diarrheal samples [33]. Recently, we performed a survey on norovirus-like gastroenteritis incidence in subjects consuming 100 mg lactoferrin-containing products including yogurt, yogurt drinks, and milk-type drinks [34]. The results indicated a lower incidence of norovirus-like gastroenteritis in groups who frequently consumed lactoferrin products compared with groups who consumed them at a lower frequency (Fig. 2). Because there is no prophylactic or therapeutic treatment for noroviral gastroenteritis, lactoferrin is a promising candidate to prevent infection and further studies are warranted to establish more reliable evidence.

4. Summer colds

Summer colds, also called summer minor illnesses, are caused by adenoviruses and a family of viruses called enteroviruses. These have a preference for warmer weather. Adenovirus mainly causes upper and lower respiratory tract infections, but also causes diseases of the intestine, eyes, liver, urinary tract and lymphoid tissue. Adenovirus is known to cause pharyngoconjunctival fever, also called pool fever. Runny nose, nasal congestion and postnasal drainage are complaints associated with both summer and winter colds. However, enteroviruses may cause more complicated illnesses, which include fever, sore throat, hacking cough, diarrhea, and skin rash. Enteroviruses, enterovirus 71 and coxsackievirus A16, are known as common causative viral agents for hand, foot, and mouth diseases in humans.

Lactoferrin inhibits the cytopathic effect of adenovirus in HEp-2 cells [35–37], where the effect of bovine lactoferrin is more potent than that of human lactoferrin (Table 1). On the other hand, another investigation reported that human lactoferrin promotes the binding of adenovirus to human corneal epithelial cells and also infection of the cells by adenovirus [38]. In this experimental system, there was no data on bovine lactoferrin. The anti-enteroviral activities of lactoferrin are indicated in poliovirus, enterovirus 71, coxsackievirus A16, echovirus 5, and echovirus 6 [39–44]. Remarkably, bovine lactoferrin induced IFN-α expression of human neuroblastoma cells (SK-N-SH) and inhibited enterovirus 71-induced interleukin (IL)-6 production [41]. The antiviral activity of bovine lactoferrin was not obvious in echovirus 9 [40].

Following enterovirus 71 infection, neonatal pups ingesting transgenic milk expressed recombinant porcine lactoferrin showed significantly higher survival rate and heavier body weight compared to wild-type mice [45] (Table 2). On the other hand, oral supplementation of bovine lactoferrin at a dose of 70 mg/day did not show beneficial effects in the prevention of enterovirus 71 or rotavirus infection in children [46].

Table 1
In vitro effects of lactoferrin against viruses causing common infections.

Disease virus	Lactoferrin species[a]	Effective dose (IC50)	Cell type	Effect	Reference
Common cold					
Respiratory syncytial virus (RSV)	hLF	10–100 μg/ml	Human epidermoid carcinoma (HEp-2)	Inhibition of virus growth	[9]
RSV	hLF	100–1000 μg/ml	HEp-2	Inhibition of virus growth	[10]
RSV	hLF	100 μg/ml	HEp-2	Reduction of virus entry into cells	[11]
Parainfluenza virus type 2 (PIV-2)	bLF		Rhesus monkey kidney ($LLCMK_2$)	Inhibition of virus entry into cells	[12]
Influenza					
Influenza A virus (H3N2)	bLF	0.89 μM	Madin–Darby canine kidney (MDCK)	Inhibition of cytopathic effect	[13]
Influenza A virus (H3N2)	Native bLF	0.625 μM	MDCK	Inhibition of cytopathic effect	[14]
	Apo-bLF	1.56 μM			
Influenza A virus (H1N1, H3N2)	bLF	25–250 pM	MDCK	Inhibition of virus replication	[15]
	bLF C-lobe	10–50 pM			
Avian influenza A virus (H5N1)	bLF	40–80 μg/ml	MDCK	Antiviral activity	[16]
	Esterified bLF	<20 μg/ml			
Viral gastroenteritis					
Rotavirus	Apo-bLF	29–58 μg/ml	Human colon adenocarcinoma (HT-29)	Inhibition of cytopathic effect	[25]
	Fe^{3+}-bLF	29–58 μg/ml			
Rotavirus	Apo-bLF	50 μg/ml	HT-29	Inhibition of cytopathic effect	[26]
	Desialylated bLF	12 μg/ml			
Feline calicivirus (FCV, norovirus surrogate)	bLF	1000 μg/ml	Crandell-Reese feline kidney (CRFK)	Inhibition of cytopathic effect	[27]
	LFcin B	50–200 μg/ml			
Murine norovirus (MNV)	bLF	5–15 μg/well	Murine macrophage (Raw264.7)	Inhibition of cytotoxic damage	[28]
Summer cold					
Adenovirus	bLF	80 μg/ml	HEp-2	Inhibition of cytopathic effect	[35]
	hLF	560 μg/ml			
Adenovirus	bLF	0.78 μM	HEp-2	Inhibition of cytopathic effect	[36]
	hLF	6.25 μM			
	LFcin B	6.25 μM			
Adenovirus	bLF			Interaction with viral III and IIIa structural proteins	[37]
Adenovirus	hLF	100 μg/ml	Human corneal epithelial (HCE)	Promotion of virus binding and infection of cells	[38]
Poliovirus (PV)	bLF	650 μg/ml	African green monkey kidney (Vero)	Inhibition of cytopathic effect	[39]
	hLF	370 μg/ml			
Enterovirus 71 (EV71)	bLF	11–25 μg/ml	Human embryonal rhabdomyosarcoma (RD)	Inhibition of cytopathic effect	[40]
	hLF	103–185 μg/ml			
EV71	bLF	34.5 μg/ml	RD and human neuroblastoma (SK-N-SH)	Inhibition of infection	[41]
Coxsackievirus A16	bLF	9.3 μg/ml	Vero	Inhibition of cytopathic effect	[40]
Echovirus 5	bLF	1000 μg/ml	Human colorectal adenocarcinoma (Caco-2)	Inhibition of virus replication	[42]
	Digested bLF	1000 μg/ml			
Echovirus 6	bLF	12.5 μM	Green monkey kidney (GMK)	Inhibition of viral infection	[43]
	bLF N-lobe	12.5 μM			
	LFcin B	12.5 μM			
Echovirus 6	bLF			Interaction with viral capsid proteins	[44]
Echovirus 9	bLF	>250 μg/ml	Vero	Inhibition of cytopathic effect	[40]
Herpes					
Herpes simplex virus-1 (HSV-1)	hLF	500 μg/ml	Human embryo lung (HEL)	Inhibition of viral infection	[47]
HSV-1	hLF	1.41 μM	Vero	Inhibition of cytopathic effect	[48]
	bLF	0.12 μM			
HSV-1	Apo-bLF	28 μg/ml	Vero	Reduction of infection	[49]
	Fe^{3+}-bLF	12 μg/ml			
HSV-2	Apo-bLF	31 μg/ml	Vero	Reduction of infection	[49]
	Fe^{3+}-bLF	5 μg/ml			
HSV-1	bLF	10 μg/ml	Vero	Inhibition of cytopathic effect	[50]
	bLF1-280	25 μg/ml			
	bLF345-689	320 μg/ml			
HSV-1	bLF	10 μg/ml	Vero	Inhibition of viral antigen synthesis	[51]
	bLF N-lobe	30 μg/ml			
	bLF C-lobe	860 μg/ml			
HSV-1	bLF	252 μg/ml	Vero	Inhibition of viral replication	[52]
	bLF with glycyrrhizic acid	15 μg/ml			
HSV-1, HSV-2	bLF		Vero	Inhibition of viral cell-to-cell spread	[53]
	hLF				
	LFcin B				
	LFcin H				
HSV-1	bLF		Vero	Inhibition of intracellular virus trafficking	[54]
	LFcin B				
HSV-2	bLF	1000 μg/ml	GMK	Inhibition of viral infection	[55]
	LFcin B	100 μg/ml			

[a] Lactoferrin species are abbreviated as follows: human lactoferrin (hLF), bovine lactoferrin (bLF), lactoferricin B (LFcin B), and lactoferricin H (LFcin H).

Table 2
Effects of orally administered lactoferrin on common viral infections.

Disease virus[a]	Lactoferrin species[b]	Dose, duration[c]	Subject, number	Method	Effect	Reference
Common cold						
ND	bLF	600 mg LF/body/d or no administration, 3 m	Human (adult woman), 398	Questionnaire survey	Reduction of common cold-like symptoms	[17]
ND	bLF and Ig-rich whey protein	400 mg LF + 200 mg Ig/body/d or placebo, 3 m	Human (adult), 105	Double blind randomized placebo-controlled trial	Reduction of cold incidence	[18]
RSV	bLF	2 to 10 mg LF/body/d or PBS, 7 d	Mice, 58	Intranasal virus infection	No difference in viral loads or disease severity	[19]
Influenza						
Influenza virus A (H1N1)	bLF	62.5 mg/body/d, 6 d	Mice, 40	Intranasal virus infection	Reduction of lung consolidation score and infiltrated leukocytes	[20]
Viral gastroenteritis						
Rotavirus	bLF	100 mg LF/body/d or no administration, 3 m	Human (children), 234	Non-randomized controlled study	Amelioration of severity of rotaviral gastroenteritis	[30]
Rotavirus and other pathogens	hLF	50 to 80 mL solution with 1 g/L hLF and lysozyme/kg or control solution, 48 h	Human (children) 140	Randomized, double-blind controlled trial	Decrease in duration and volume of diarrhea, but no difference in rotaviral incidence	[31]
Norovirus	bLF	400 mg LF/body/d or no administration, 4 m	Human (children), 91	Randomized controlled study	Reduction of noroviral gastroenteritis incidence	[32]
Norovirus and other pathogens	bLF	500 mg LF twice/d or placebo, 6 m	Human (children) 555	Randomized, double-blind controlled trial	Reduction of diarrhea longitudinal prevalence, but no difference in noroviral incidence	[33]
Norovirus	bLF	100 mg LF/body/d at 1–7 times per w, one winter season	Human, 461	Questionnaire survey	Lower incidence of noroviral gastroenteritis in frequently consuming groups	[34]
Summer cold						
EV71	pLF	Milk of wild type or pLF-transgenic mice, 3 w	Mice (neonate), 30	Intraperitoneal virus infection	Increase in survival rate and body weight	[45]
EV71 and rotavirus	bLF	70 to 85 mg LF/body/d or no administration, 15 m	Human (children), 172	Randomized, single blind trial	No difference in incidence of enterovirus or rotavirus infection	[46]
Herpes						
HSV-1	bLF	1.5% bLF solution in drinking water, 20 d	Mice, 30	Cutaneous viral infection	Prevention of body weight loss and increase in cytokine responses	[56]

[a] ND indicates that virus species were not determined.
[b] Lactoferrin species are abbreviated as follows: bovine lactoferrin (bLF), human lactoferrin (hLF), and porcine lactoferrin (pLF).
[c] Duration is abbreviated as follows: hours (h), days (d), weeks (w), and months (m).

5. Herpes

Herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) establish life-long latent infections in the host and can re-emerge periodically throughout life, primarily causing facial and genital herpetic lesions, respectively. The in vitro anti-herpes activities of lactoferrin have been studied in HSV-1 [47–54] and HSV-2 [49,53,55] (Table 1).

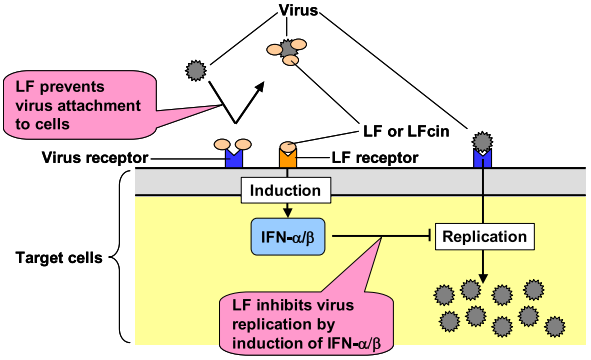

Fig. 1. Deduced mechanism of antiviral effect of lactoferrin. Lactoferrin (LF) or lactoferricin (LFcin) prevents virus attachment to the target cells by binding to the virus receptor on the target cells or binding to the virus. In addition, lactoferrin induces IFN α/β production and thereby inhibits virus replication after entry of the virus into the cells.

The effect of orally administered lactoferrin in HSV infection has been reported in one study [56] (Table 2). This study indicated that lactoferrin administration prevents body weight loss and increases the production of Th1 cytokines, including IFN-γ, IL-12, and IL-18, after HSV-1 cutaneous infection in mice. These enhanced Th1 cytokine responses may help host protection against HSV-1 infection.

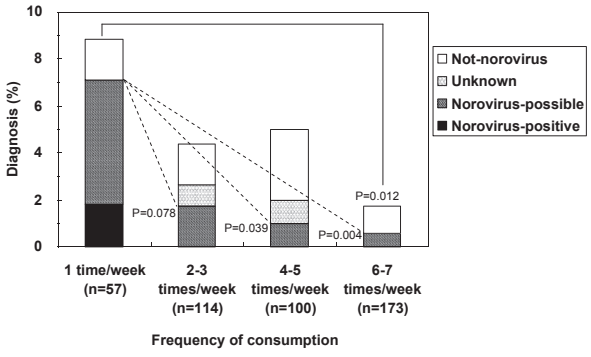

Fig. 2. Diagnosis of noroviral gastroenteritis in subjects who consumed lactoferrin-containing products and who visited the clinic. Subjects were divided by frequency of consumption of lactoferrin-containing products, and by population of diagnosis by the medical doctor, (not-norovirus, unknown, norovirus-possible, and norovirus-positive in the testing, are indicated). Proportions of subjects visited the clinic or diagnosed as norovirus-possible/positive were statistically compared between each consumption frequency group and the group of 1 time/week consumption, and *p*-values were indicated.

6. Conclusions

Lactoferrin exhibits inhibitory activities against a wide range of viruses in vitro. The effects of lactoferrin oral administration have been studied in various viral infections in animals and humans. These infections included life-threating chronic hepatitis C [57], but no significant efficacy of lactoferrin was demonstrated in a clinical study with a relatively large number of patients [58]. On the other hand, the beneficial effects of lactoferrin have recently been found in common viral infections including the common cold, influenza, viral gastroenteritis, summer cold, and herpes. As lactoferrin is a food component, it is easily consumed by an individual to prevent these infections. Although the mechanism of action of lactoferrin has not been fully elucidated, direct antiviral activities exerted in the gastro-intestinal tract and systemic immune-modulation seem to be involved in these effects. Further basic and clinical studies will clarify the usefulness of lactoferrin in this field.

Conflict of interest

All authors are employees of Morinaga Milk Industry.

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